THE CROP JOURNAL 4 (2016) 290–303

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Accuracy of genomic selection in biparental populations of flax (*Linum usitatissimum* L.)

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ARTICLE INFO

Article history:
Received 13 December 2015
Received in revised form
9 March 2016
Accepted 15 March 2016
Available online 31 March 2016

Keywords:
Genomic selection
SSR
Flax
Linseed
Seed yield
Fatty acid composition

ABSTRACT

Flax is an important economic crop for seed oil and stem fiber. Phenotyping of traits such as seed yield, seed quality, stem fiber yield, and quality characteristics is expensive and time consuming. Genomic selection (GS) refers to a breeding approach aimed at selecting preferred individuals based on genomic estimated breeding values predicted by a statistical model based on the relationship between phenotypes and genome-wide genetic markers. We evaluated the prediction accuracy of GS (r_{MP}) and the efficiency of GS relative to phenotypic selection (*RE*) for three GS models: ridge regression best linear unbiased prediction (RR-BLUP), Bayesian LASSO (BL), and Bayesian ridge regression (BRR), for seed yield, oil content, iodine value, linoleic, and linolenic acid content with a full and a common set of genome-wide simple sequence repeat markers in each of three biparental populations. The three GS models generated similar r_{MP} and *RE*, while BRR displayed a higher coefficient of determination (R^2) of the fitted models than did RR-BLUP or BL. The mean r_{MP} and *RE* varied for traits with different heritabilities and was affected by the genetic variation of the traits in the populations. GS for seed yield generated a mean *RE* of 1.52 across populations and marker sets, a value significantly superior to that for direct phenotypic selection. Our empirical results provide the first validation of GS in flax and demonstrate that GS could increase genetic gain per unit time for linseed breeding. Further studies for selection of training populations and markers are warranted.

1. Introduction

Genomic or genome-wide selection (GS) is a breeding method based on the relationship between phenotype and a genome-wide set of genetic markers. A practical GS approach in breeding includes several steps [1–3]: (1) construction of an optimal training population that is genetically diverse and large; (2) phenotyping individuals of the training population in multiple environments; (3) genotyping individuals of the training population with a genome-wide set of genetic markers; (4) fitting an optimal statistical model based on the phenotypic and genotypic data, and estimating marker effects in the model; (5) genotyping test individuals with the markers used in GS model fitting; and (6) applying the GS model to

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Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.

http://dx.doi.org/10.1016/j.cj.2016.03.001

estimate genomic estimated breeding values (GEBVs) of test individuals for selection. GS has been proposed to be superior to conventional phenotypic selection and maker assisted selection (MAS) in time and money savings, and thereby to increase the efficiency of plant breeding [4].

GS is commonly applied in animal breeding [2], and extensive studies of GS in plants have been performed since 2007. Two research approaches have been employed to evaluate the efficiency of GS in plant breeding. The first is based on simulated [5–10] or real [11–13] marker data with simulated population data. Computer simulation is advantageous for generating data based on strict assumptions and for investigating the relationship of GS accuracy with different levels of factors influencing GS such as population type, marker density, linkage disequilibrium of populations, QTL number, and population size. The second approach is to use empirical data, a practical approach to demonstrating actual GS efficiency in plant breeding. To date, results of evaluation of GS accuracy have been reported for several annual crops such as maize [14–23], barley and *Arabidopsis* [14], wheat [4,16], rice [24–26], sugar beet [27], and sugarcane [28], and perennial trees such as loblolly pine, eucalyptus [29–31], and apple [32].

For millennia, flax has been used as a food source and to produce durable fibers and linen. More recently, flax has become an important multi-purpose crop, owing to an increasing demand for both oil and fiber [33]. Flax seeds typically contain 35–50% oil composed of five main fatty acids: palmitic (PAL, 6.0%), stearic (STE, 2.5%), oleic (OLE, 19.0%), linoleic (LIO, 13.0%), and linolenic (LIN, 55.0%) [34,35]. LIN is also referred to as α-linolenic acid (ALA). Recent work has shown that flax's omega-3 fatty acids (LIN) and plant estrogens contribute to reducing blood cholesterol levels and mitigate heart disease and certain cancers in humans [36–38]. The major breeding aims of linseed development are high seed yield (YLD), high oil content (OIL), and high (>65%), or low (2–4%) LIN content. The first registered high-LIN linseed cultivar in Canada is NuLin 50 with 68% LIN (http://www.viterra.ca) [39]. Also, low-LIN (2–4%) and high-LIO (65–70%) cultivars have been obtained by mutation breeding [39–41]. High-LIN flaxseed is one of the richest dietary sources of ALA and is also a good source of soluble fiber mucilage [42], whereas low LIN in seeds will effectively improve the oxidative stability and suitability of linseed oil for food uses [43]. Flax straw and its processed forms are widely used in the manufacturing of fine papers and some industrial fiber products such as the interior paneling of vehicles (http://www.flaxcouncil.ca/english/index.jsp). The major breeding aims of fiber flax are increased straw yield, fiber content in straw, fiber quality, and resistance to disease and abiotic stresses. However, phenotyping of seed yield, seed quality, and fiber traits is time-consuming, labor-intensive, and consequently costly. In addition, most of these traits are quantitative. Conventional breeding using phenotyping continues to predominate in flax breeding programs. Even MAS based on single QTL can be ineffectual because of potential overestimated QTL effects and small proportions of the genetic variation explained by the QTL [3]. By predicting breeding values of these traits for selection without prior phenotyping, GS provides an alternative approach to the quantitative traits in flax breeding.

Single sequence repeats (SSRs) or microsatellites are stretches of DNA containing a variable number of short tandem repeats. They are generally codominant, highly polymorphic, abundant, and reliable, and can be readily developed from existing genomic sequences or expressed sequence tags (ESTs) [44]. Currently more than 1400 SSR markers have been developed in flax from EST libraries [45–47] or genomic sequences [45,48–51]. These SSR markers have been used for the construction of genetic maps [52,53], genetic diversity assessment [54,55], QTL mapping [53], and association studies [56,57]. A total of 770 markers were incorporated into a consensus map from three biparental populations. The map had a total length of 1551 cM with a mean marker density of one marker every 2 cM and covered an estimated 74% of the predicted flax genome size of 370 Mb [52]. Thus, these SSR markers span most regions of the flax genome. Using the same set of SSR markers, Cloutier et al. detected two major QTL each for LIO, LIN, and iodine value (IOD), and one major QTL for PAL, in a doubled-haploid (DH) population of 78 lines generated from a cross between SP2047 and UGG5-5 [53]. Soto-Cerda et al. [56] used the association mapping approach and a flax core collection of 390 accessions with 460 SSR markers to identify QTL for seed quality traits in this germplasm collection. A total of nine QTL were associated with OIL, LIO, and LIN, some of which colocalized with QTL previously identified in the SP2047/UGG5-5 biparental population [53]. These previous studies provide data useful for validating results from GS in flax.

The objective of this study was to explore the feasibility of GS in flax breeding by comparing accuracies and relative efficiencies of genomic prediction in multiple biparental populations using genome-wide SSR marker sets and several GS statistical models for seed yield and seed quality traits in flax.

2. Materials and methods

2.1. Populations

Three biparental populations were used for evaluation of GS. The first population (BM) was generated by single-seed descent from a cross between CDC Bethune [58] and Macbeth [59], and consisted of 243 F_6-derived recombinant inbred lines (RILs). Its two parents were high-yielding Canadian linseed cultivars containing 55–57% LIN [58,59]. The second population (EV) comprised 90 F_6-derived RILs from a cross between E1747, an ethyl methane sulphonate (EMS)-induced low LIN breeding line [60], and Viking, a French fiber flax cultivar grown widely in 2000 but deregistered in 2012. The third population (SU) was an F_1-derived doubled haploid (DH) population of 78 lines obtained from a cross between the breeding line SP2047, which gave rise to a yellow-seeded Solin variety called Linola 2047 that contains only 2–3% LIN, and breeding line UGG5-5, which is a high-LIN line with 63–66% LIN [53,61]. These three populations have been used for genetic mapping and QTL detection [52,53,62].

2.2. Phenotypic data

Lines from the three biparental populations were evaluated in field tests over 3 or 4 years (2009–2012) at two sites (Morden,

Manitoba, and Kernen Crop Research Farm near Saskatoon, Saskatchewan) in Canada. A type-2 modified augmented design (MAD2) [63] was used for the field experiments from which phenotypic data were collected. The detailed experimental design was described in [64]. All 243 lines of the BM population were genotyped in eight environments (2009–2012 at two sites), and 86 lines of the EV population and 72 lines of the SU population were evaluated in six environments (2010–2012 at two sites). Of the lines evaluated in field tests, 243 lines in BM, 86 in EV, and 70 in SU were genotyped with SSR markers and used for GS evaluation.

Seed yield data were recorded by harvesting two 0.5-m sections from rows located in the central part of each subplot. A total of 1 g of seed from each line in each environment was sampled for measurement of oil content and fatty acid composition. Methyl esters of fatty acids were prepared according to the American Oil Chemists' Society (AOCS) Official Method Ce 2-66 and fatty acid composition was measured by capillary gas chromatography (GC) following AOCS Official Method Ce 1e-91 [65]. Oil content was determined by nuclear magnetic resonance calibrated against the Federation of Oils, Seeds and Fats Associations (FOSFA) extraction reference method. Five target traits in flaxseed breeding including YLD (t ha^{-1}), OIL (%), IOD, LIO (%), and LIN (%) were selected for GS evaluation.

2.3. Genotyping data

In this GS assessment, 340, 443, and 474 polymorphic SSR markers were used for BM, EV, and SU, respectively. These markers have been incorporated into respective linkage groups for each of the three populations and into a consensus linkage map of all three populations [52]. The consensus genetic map covers 74% of the estimated flax genome size. Thus, these SSR markers represent a genome-wide set of SSR markers. To compare effects of genomic predictions on marker sets of different sizes, 102 markers extracted from the full marker set and shared by all three populations were used as a common marker set. Thus, for each of the three populations, we compared two marker sets, the full and the common. For a few missing genotypes in the data sets, the EM algorithm implemented in the R (version 2.5, http://cran.r-project.org/) package rrBLUP [66] was used for data imputation [67].

2.4. Statistical models used for GS

Three predictive models for GS were compared: ridge regression best linear unbiased prediction (RR-BLUP) [3,68], Bayesian LASSO (BL) [69], and Bayesian ridge regression (BRR) [70]. All predictive models estimate marker effects by modelling markers as random effects. The average performance of traits in multiple environments was used to represent phenotypes. No fixed effects were fitted in the models. The statistical models and computation procedures have been described in detail [71,72]. The R package rrBLUP [66] was used to fit the RR-BLUP model, and the R package BLR [73] was used to fit the BL and BRR models. The parameters used for fitting BL and BRR were determined based on suggestions of de los Campos et al. (2013) [73]. Broad-sense heritability of traits estimated in the three populations (see Statistical analysis) was used for building the BL and BRR models.

2.5. Evaluation of GS

Fivefold cross-validation was used to evaluate the accuracy of GS within the three single biparental populations and their pooled populations. The respective data set was randomly partitioned into five subsets. For a given partition, each fold (subset) was in turn used for validation (test data set), and the remaining four of the five subsets were used as a training data set. This partitioning of training and test data sets was repeated 100 times. In this manner, a total of 500 training data sets were formed to build GS models and estimate marker effects, which then were used to predict the breeding values of the lines in the 500 test data sets using the same set of markers. Each of the sampling data sets was used for GS modeling, GEBV prediction, and evaluation of all studied GS models. The accuracy of the genomic predictions (r_{MP}) was defined as the Pearson's correlation between the genetic values predicted by GS and the observed phenotypic values. The relative efficiency of genomic prediction over phenotypic selection (*RE*) was estimated as $r_{MP}/\widehat{H}^2$ [20,74], where $\widehat{H}^2$ refers to the broad-sense heritability described in the next section. The mean r_{MP} and *RE* of the total 500 samplings for a combination of a marker set, a GS model, and a population were used to describe, respectively, the prediction accuracy of GS and efficiency of one cycle of GS relative to one cycle of phenotypic selection for a trait. In addition, the coefficient of determination (R^2) for each fitted GS model was estimated. The R^2 of a fitted GS model was calculated as $1 - SS_{res}/SS_{total}$, where SS_{res} is the sum of squares of the residuals and SS_{total} is the total sum of squares, representing the ratio of the variance accounted for by the model to the total variance of a trait. R^2 represents the goodness of fit of a GS model that establishes a relationship between phenotypes and genotypes of a training population. For comparisons of different GS models, marker sets, traits, or biparental populations, a joint analysis of variance (ANOVA) with the PROC ANOVA procedure of SAS was performed to test the statistical significance of differences in r_{MP}, *RE*, and R^2.

2.6. Statistical analysis

All phenotypic observations from the field trials and laboratory measurements were adjusted for soil heterogeneity as previously described based on the MAD2 pipeline [64]. The adjusted phenotypic data of each biparental population was analyzed separately using a linear model:

$$y_{ij} = \mu + G_i + E_j + (GE)_{ij} + \varepsilon_{ij},$$

where y_{ij} is the adjusted value of the *i*-th genotype (line) ($i = 1, 2, \ldots, g$) in the *j*-th environment (a combination of year and site) ($j = 1, 2, \ldots, e$); μ is the overall mean; G_i is the genotype effect of the *i*-th genotype; E_j is the environment effect of the *j*-th environment; $(GE)_{ij}$ is the interaction effect between the *i*-th genotype and the *j*-th environment; and ε_{ij} is the joint experimental error estimated based on the joint ANOVA of three control cultivars in the MAD2 design [75].

Broad-sense heritability (H^2) of a trait was estimated as $\widehat{H}^2 = \hat{\sigma}_G^2 / \hat{\sigma}_P^2$, where $\hat{\sigma}_G^2$ and $\hat{\sigma}_P^2$ are the genetic and phenotypic variance, respectively. For the $\widehat{H}^2$ on a plot basis across environments, $\hat{\sigma}_P^2 = (\hat{\sigma}_G^2 + \hat{\sigma}_{GE}^2 + \hat{\sigma}_e^2)$, where $\hat{\sigma}_G^2$, $\hat{\sigma}_{GE}^2$, and $\hat{\sigma}_e^2$ correspond to the genetic variance, the genotype and environment interaction (G × E) variance, and the error variance, respectively. But for the $\widehat{H}^2$ on an entry-mean basis, $\hat{\sigma}_P^2 = \hat{\sigma}_G^2 + \hat{\sigma}_{GE}^2/e + \hat{\sigma}_e^2/(en)$, where e and n represent, respectively, the number of environments and the replications per environment [75]. The genetic correlation coefficients ($\hat{r}_G$) between any two traits were estimated as $\hat{r}_G = \widehat{COV}_{G12}/\sqrt{\hat{\sigma}_{G1}^2\hat{\sigma}_{G2}^2}$, where $\widehat{COV}_{G12}$ is the genetic covariance between two traits, and $\hat{\sigma}_{G1}^2$ and $\hat{\sigma}_{G2}^2$ are their respective genetic variances. The genetic coefficient of variation (GCV) was calculated as $\widehat{GCV} = \hat{\sigma}_G/\bar{x}$, where $\bar{x}$ is the population mean. These variance and covariance components were estimated using the method of moments based on ANOVA or multivariate ANOVA (MANOVA) [75] and on restricted maximum likelihood (REML) [76,77]. The standard error of $\widehat{H}^2$ was estimated using the delta method implemented for the MAD2 design [75]. The four methods for estimating $\widehat{H}^2$, ANOVA on a plot basis (AP), ANOVA on an entry-mean basis (AM), REML on a plot basis (RP), and REML on an entry-mean basis (RM) were compared, and the AP was finally adopted.

All statistical analyses were performed using R, SAS (V9.3, SAS Institute, Inc., Cary, NC, USA), and custom Perl scripts.

3. Results

3.1. Genetic variation of biparental populations

Genetic variation of the five traits YLD, OIL, IOD, LIO, and LIN in the three biparental populations was first assessed. Separate ANOVAs for the five traits in the three populations showed that all traits had significant genetic variation (Table S1). Although all traits showed large variation across environments (3 or 4 years at two sites), the G × E interactions showed a rather small proportion of the total variation for IOD, LIO, and LIN in all three populations (Table S1) and some of them were not statistically significant, indicating that, for these traits, the performances of the lines within each of the three biparental populations had relatively consistent ranks in the different environments. YLD had a large proportion of the G × E variance over the total variance, whereas OIL was less affected by environment than was YLD.

Although the five traits showed marked genetic differences within the three populations, their population means and genetic variances varied across populations as a consequence of parental differences (Fig. 1, Table 1). Among the

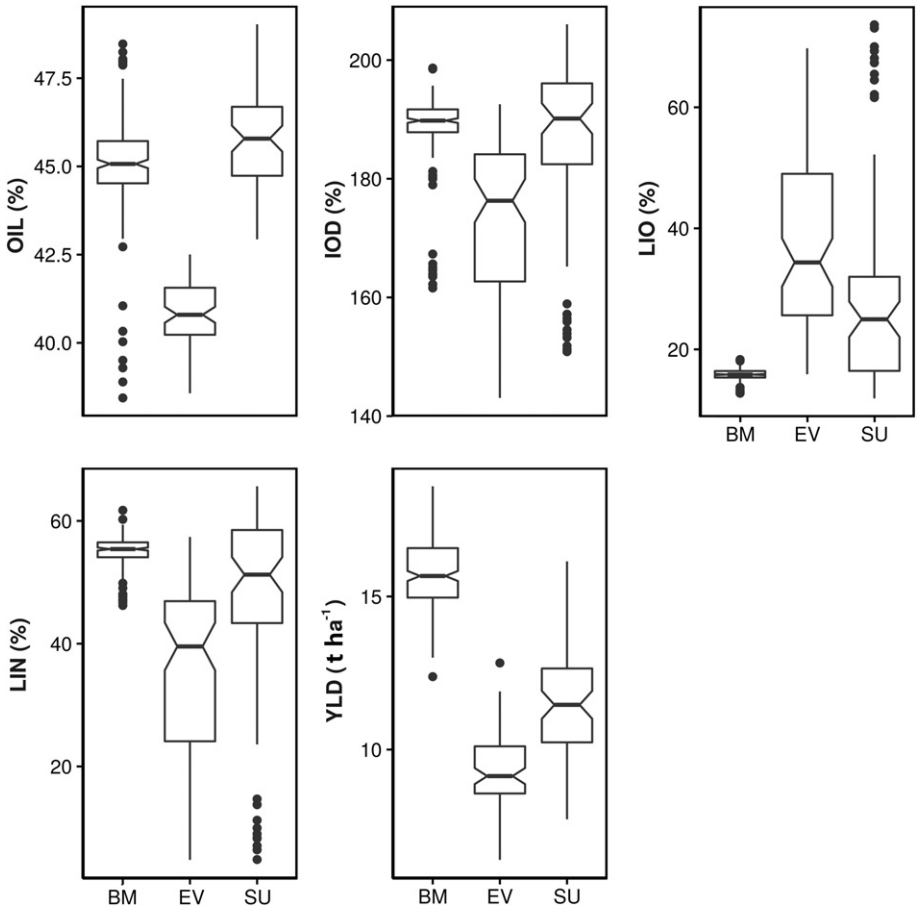

Fig. 1 – Box-and-whisker plots for phenotypic performance of five traits in biparental populations BM, EV, and SU.

Table 1 – Estimates of genetic parameters of five traits in three biparental populations.

Trait	Pop	Mean	$\widehat{H}^2 \pm s$	$\widehat{\sigma}^2_P$	$\widehat{\sigma}^2_G$	$\widehat{\sigma}^2_{GE}$	$\widehat{\sigma}^2_e$	$\widehat{GCV}$ (%)
OIL (%)	BM	45.03	0.38 ± 0.02**	2.20	0.84	0.89	0.46	2.03
	EV	40.76	0.57 ± 0.04**	1.40	0.80	0.04	0.56	2.19
	SU	45.78	0.68 ± 0.04**	2.22	1.52	0.44	0.26	2.69
IOD	BM	188.83	0.68 ± 0.02**	12.76	8.66	2.56	1.54	1.56
	EV	172.44	0.92 ± 0.01**	227.36	208.12	4.95	14.29	8.37
	SU	185.53	0.92 ± 0.01**	246.27	226.92	0.00	19.44	8.12
LIO (%)	BM	15.88	0.65 ± 0.02**	1.15	0.74	0.24	0.16	5.43
	EV	37.45	0.91 ± 0.01**	277.98	252.97	5.10	19.91	42.47
	SU	30.26	0.94 ± 0.01**	336.56	314.82	4.94	16.81	58.64
LIN (%)	BM	55.10	0.67 ± 0.02**	4.39	2.96	0.86	0.57	3.12
	EV	35.33	0.92 ± 0.01**	277.86	254.45	5.61	17.80	45.15
	SU	45.61	0.93 ± 0.01**	319.68	297.85	2.31	19.52	37.84
YLD (t ha^{-1})	BM	15.68	0.01 ± 0.01	12.89	0.15	8.99	3.75	2.48
	EV	9.21	0.16 ± 0.04**	5.59	0.91	0.76	3.93	10.35
	SU	11.45	0.14 ± 0.04**	10.48	1.43	4.14	4.91	10.45

$\widehat{H}^2$, $\widehat{\sigma}^2_P$, $\widehat{\sigma}^2_G$, $\widehat{\sigma}^2_{GE}$, $\widehat{\sigma}^2_e$, and $\widehat{GCV}$ are estimates of broad-sense heritability, phenotypic variance, genetic variance, genotype × environment interaction variance, error variance, and genetic coefficient of variation, respectively. *s*: standard error. All variance components and $\widehat{H}^2$ were estimated using the method of moments of ANOVA on a plot basis. BM: CDC Bethune/Macbeth; EV: E1747/Viking; SU: SP2047/UGG5-5; OIL: oil content; IOD: iodine value; LIO: linoleic acid content; LIN: linolenic acid content; YLD: seed yield. The population (Pop) sizes for the three populations BM, EV, and SU were 243, 86, and 70, respectively.

** Significance of heritability at the 0.01 probability level using approximate Z test.

three populations, SU showed the largest genetic variance, whereas BM showed the smallest but also showed a larger proportion of error variances over the total variance in the traits (Table 1). In BM, both parents, CDC Bethune and Macbeth showed relatively high LIN contents and high seed yield [58,59], resulting in a high LIN:LIO ratio and high seed yield with small genetic variation (1.56–5.43% of $\widehat{GCV}$) in the population compared with those of EV and SU (2.19–58.64% of $\widehat{GCV}$) (Table 1). Principal components analysis (PCA) of the five traits further showed that the BM individuals formed a tighter cluster than the SU and EV lines (Fig. 2). The individuals from the three populations grouped independently in different regions with little overlap, revealing their distinct underlying genetic characteristics and thus their utility for evaluation of GS in different breeding populations.

Broad-sense heritability $\widehat{H}^2$ of traits on a plot basis was estimated in the three biparental populations separately (Table 1). $\widehat{H}^2$ represents the ratio of genetic variance to total phenotypic variance of a trait and depends largely on genetic coefficient of variation, G × E interaction, and error variance of the trait. Because of low genetic variation and compared to the error variance for most traits in BM, the $\widehat{H}^2$ values for five

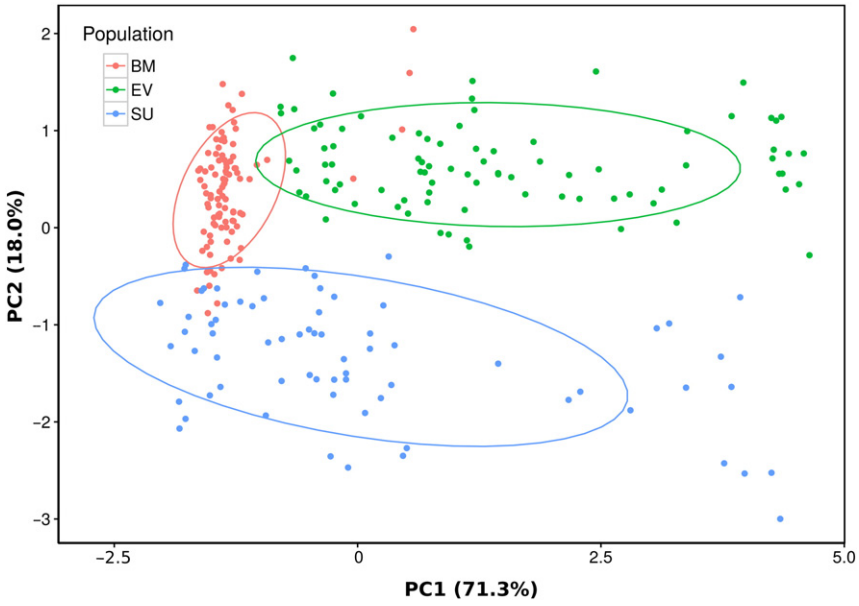

Fig. 2 – Principal components analysis of 399 lines in three biparental populations (BM, EV, and SU) based on five traits across six or eight environments. The first and second principal components are plotted. The percentages in parentheses in the axis titles represent the variance explained by each of the two principal components.

traits were lower than those in EV and SU, with the $\widehat{H}^2$ for YLD not significantly different from zero (Table 1). Based on the heritability estimates in EV and SU, LIN, LIO, and IOD had high $\widehat{H}^2$ values (>0.90), OIL had intermediate $\widehat{H}^2$ values (0.5–0.7), and YLD had low $\widehat{H}^2$ values (<0.2) because of the higher error and the G × E variance associated with this trait (Table 1). Owing to the relatively small proportions of the error and G × E variances in the total variance, the $\widehat{H}^2$ values for LIN, LIO, and IOD were attributed largely to the genetic variance of populations, whereas YLD and OIL were significantly affected by G × E interaction.

Genetic correlation coefficients among the five traits were calculated separately for the three populations (Table 2, upper triangle). The results showed that estimates of genetic correlations between traits were also affected by the genetic characteristics of the populations. Genetic correlation coefficients between some traits were inconsistent in quantity and/or direction of correlation among the biparental populations. As expected, IOD, an estimate of the desaturation level of oil, was highly negatively (close to −1) correlated with LIO and positively (close to 1) correlated with LIN in both EV and SU. However, significant positive correlations between IOD and both LIO (0.13) and LIN (0.96) were observed in BM. OIL showed varying correlations with some traits in the three biparental populations. OIL was correlated negatively with LIN and positively with LIO in SU, but no correlation between these three traits was observed in the other two populations. Between YLD and OIL, a highly significantly positive genetic correlation in EV but a negative one in SU was obtained.

3.2. Accuracy of genomic prediction

Accuracies of genomic prediction (hereafter called prediction accuracies or r_{MP}) in the three biparental populations (BM, EV, and SU) and the two SSR marker sets (full and common) using three GS models (RR-BLUP, BL, and BRR) for five seed traits were estimated based on fivefold cross-validation with 100 replications. A joint ANOVA of r_{MP} showed significant differences among the five traits, among the three populations, and between the two SSR marker sets (Table S2). Significant interactions between these three factors (traits, populations, and marker sets) were also observed, such as population × marker set, population × trait, and marker set × trait (Table S2).

The mean r_{MP} of 500 samplings for each trait–population–marker set–GS model combination was calculated to describe the predicted accuracy (Table 3). Overall mean r_{MP} for all five traits ranged from 0.25 to 0.52 for the full marker set and from 0.19 to 0.41 for the common marker set. On average, higher r_{MP} estimates were obtained for IOD, LIO, and LIN (0.41, 0.45, and 0.47, respectively) than the other traits because of their higher $\widehat{H}^2$, $\hat{\sigma}^2_G$, or $\widehat{GCV}$. OIL had a lower mean r_{MP} (0.37) than IOD, LIO, and LIN. Owing to the low $\widehat{H}^2$ for YLD, a low mean r_{MP} (0.22) was obtained (Table 3). The r_{MP} estimates of the traits using the full marker set (0.44) were consistently and significantly superior to those using the common marker set (0.33) (Table 3, Fig. 3A). Among the three biparental populations, the overall mean r_{MP} of the five traits within EV (0.47) was significantly higher than that within SU (0.35), which was also significantly superior to that within BM (0.32) (Fig. 3B).

Although a significant difference in r_{MP} between the three GS models at the 0.05 probability level was observed (Table S1), BL, BRR, and RR-BLUP had similar mean r_{MP} in the full marker set (0.33–0.34) and in the common marker set (0.43–0.44) (Fig. 4). The overall mean r_{MP} for BL, BRR, and RR-BLUP were 0.39, 0.38, and 0.38, respectively, showing no difference between BRR and RR-BLUP. The differences among the three GS models were much smaller than those among the populations and between the marker sets (Table S2).

The three single biparental populations were merged to generate four pooled populations to assess the effect on r_{MP} of increased genetic diversity and size of training population.

Table 2 – Genetic correlation coefficients among five traits (upper triangle) and Pearson's correlation coefficients of prediction accuracies (r_{MP}) among the same five traits (lower triangle).

Trait	Population	OIL (%)	IOD	LIO (%)	LIN (%)	YLD (t ha^{-1})
OIL (%)	BM		−0.20**	−0.01	−0.21**	0.00
	EV		−0.07	−0.01	−0.03	0.76**
	SU		−0.64**	0.65**	−0.65**	−0.35**
IOD	BM	−0.05**		0.13**	0.96**	0.06
	EV	0.29**		−0.99**	1.00**	−0.09
	SU	0.15**		−0.95**	0.99**	0.12**
LIO (%)	BM	0.05**	0.11**		0.13**	−0.20**
	EV	0.31**	0.98**		−1.00**	−0.09
	SU	0.14**	0.93**		−0.99**	−0.30**
LIN (%)	BM	0.02	0.86**	0.00		0.12**
	EV	0.30**	0.99**	0.99**		0.06
	SU	0.15**	0.97**	0.98**		0.21**
YLD (t ha^{-1})	BM	−0.07**	0.05**	−0.09**	0.07**	
	EV	0.39**	0.19**	0.20*	0.19**	
	SU	0.02	0.01	0.02	0.02	

See Table 1 for the abbreviations of traits and populations. The sample sizes for genetic correlation of phenotypic values in BM, EV, and SU were 243, 86, and 70, respectively. The sample size for Pearson's correlation of r_{MP} was 3000 for all populations. The results of the RR-BLUP model applied to the full marker set were used for calculation of Pearson's correlation coefficients.

* Statistical significance at the 0.05 probability level.

** Statistical significance at the 0.01 probability level.

Table 3 – Prediction accuracies (r_{MP}) and relative efficiency of genomic selection over phenotypic selection (*RE*) of five traits for two marker sets (full and common) in three biparental populations (BM, EV, and SU).

Trait	Population	Full		Common	
		$r_{MP} \pm s$	$RE \pm s$	$r_{MP} \pm s$	$RE \pm s$
OIL (%)	BM	0.43 ± 0.11 b	1.13 ± 0.29** a	0.37 ± 0.12 a	0.95 ± 0.30** a
	EV	0.56 ± 0.15 a	0.99 ± 0.27 b	0.29 ± 0.19 b	0.51 ± 0.33** b
	SU	0.31 ± 0.21 c	0.45 ± 0.31** c	0.25 ± 0.22 c	0.36 ± 0.32** c
IOD	BM	0.28 ± 0.14 c	0.41 ± 0.20** c	0.24 ± 0.12 c	0.35 ± 0.18** c
	EV	0.70 ± 0.11 a	0.76 ± 0.12** a	0.48 ± 0.18 a	0.52 ± 0.20** a
	SU	0.40 ± 0.19 b	0.44 ± 0.20** b	0.37 ± 0.19 b	0.40 ± 0.21** b
LIO (%)	BM	0.36 ± 0.11 c	0.56 ± 0.17** b	0.30 ± 0.18 c	0.11 ± 0.16** c
	EV	0.70 ± 0.11 a	0.77 ± 0.12** a	0.47 ± 0.18 a	0.52 ± 0.20** a
	SU	0.46 ± 0.18 b	0.50 ± 0.20** c	0.39 ± 0.19 b	0.41 ± 0.21** b
LIN (%)	BM	0.43 ± 0.12 b	0.64 ± 0.18** b	0.38 ± 0.11 b	0.56 ± 0.17** a
	EV	0.70 ± 0.11 a	0.76 ± 0.12** a	0.48 ± 0.18 a	0.52 ± 0.20** b
	SU	0.43 ± 0.19 b	0.47 ± 0.20** c	0.38 ± 0.19 b	0.41 ± 0.21** c
YLD (t ha^{-1})	BM	0.22 ± 0.11 c	–†	0.23 ± 0.11 b	–†
	EV	0.25 ± 0.19 b	1.52 ± 1.15** b	0.06 ± 0.23 c	0.39 ± 1.40** b
	SU	0.29 ± 0.23 a	2.09 ± 1.69** a	0.29 ± 0.22 a	2.09 ± 1.59** a

See Table 1 for the abbreviations of traits and populations. For each trait, the same letters represent a lack of statistical significance at the 0.05 probability level between populations. Duncan's multiple-range test was used. The *RE* estimate for YLD in BM is not available because of the non-significant heritability of YLD in BM. *s*: standard deviation.

** Significant difference of *RE* from 1.00 at the 0.01 probability level.

† *RE*s were not calculated because the estimated genetic variances were negligible.

The common marker set with the RR-BLUP model was used. Fig. 5 shows comparisons of mean r_{MP} estimates in the five traits in terms of three single populations and their pooled populations. Significant improvement of genomic prediction was achieved in some of the pooled populations, especially in EV + SU, which had large genetic variation. Significant increases in r_{MP} occurred primarily in traits with low or intermediate heritability, including YLD and OIL. For example, the mean r_{MP} for YLD reached as high as 0.50 in BM + SU and 0.47 in EV + SU, whereas that for OIL was 0.67 in EV + SU.

3.3. *RE of GS over phenotypic selection*

The mean *RE* of GS over direct phenotypic selection was calculated for the five traits (Table 3). Because the $\widehat{H}^2$ for YLD in BM was very small (0.01), and not significantly different from zero, the *RE* estimate was not reliable and was accordingly removed. Direct phenotypic selection for a trait was considered to have a baseline efficiency of 1.00. Thus, an *RE* greater than 1.00 indicates that GS is more efficient than direct phenotypic selection. Similarly with r_{MP}, significant

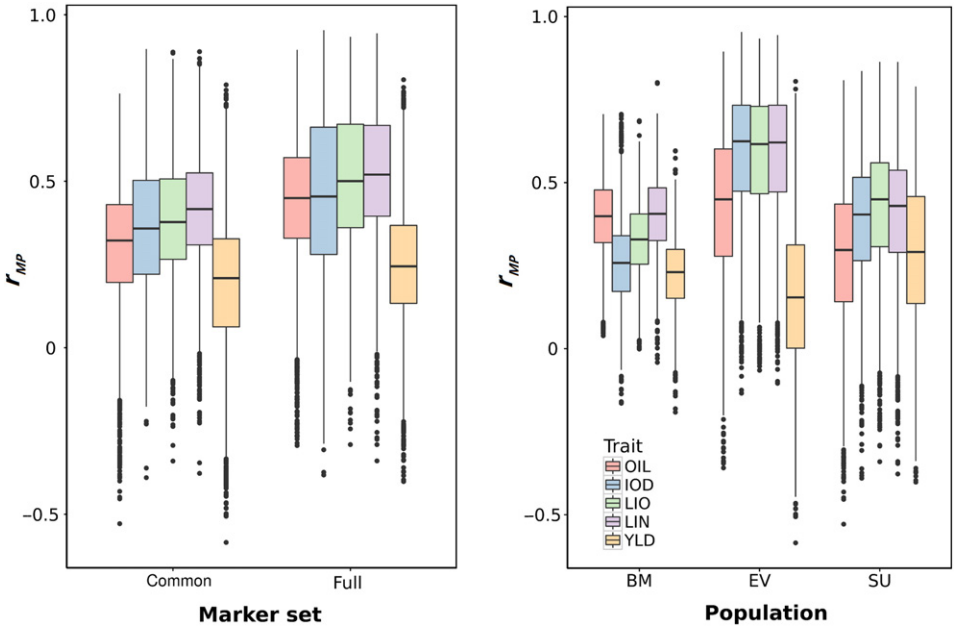

Fig. 3 – Box-and-whisker plots for the prediction accuracies (r_{MP}) of five traits assessed using fivefold cross-validation in two marker sets (A) and three biparental populations (B).

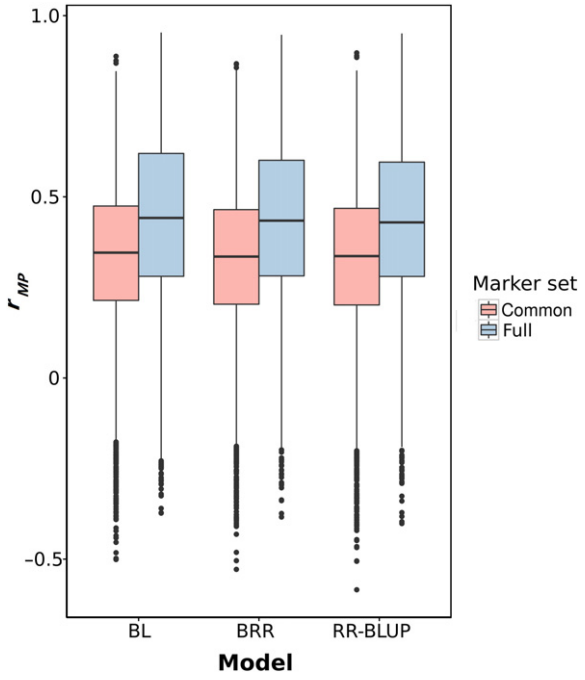

Fig. 4 – Box-and-whisker plot for prediction accuracies (r_{MP}) assessed using fivefold cross-validation in several GS models and marker sets.

differences in *RE* were observed between the two marker sets, among the three populations, and among the five traits (Table S2), but no significant difference was observed among the three GS models. Except for YLD for the common marker set in the EV population, the *RE* for YLD ranged from 1.52 to 2.09, significantly greater than 1.00 ($p < 0.01$). The overall mean *RE* values were 0.73, 0.48, 0.54, 0.56, and 1.52 for OIL, IOD, LIO, LIN, and YLD, respectively.

3.4. Goodness-of-fit of GS models

R^2 of the fitted models was calculated to show the proportion of the total variation for which a predictive model using different marker sets and different GS modeling methods could account. Statistically significant correlation between r_{MP} and model R^2 was observed ($r = 0.27$, $p = 0$). The BRR model had a significantly ($p < 0.001$) higher model R^2 than the other two (Table 2S, Fig. 6). The predictive models using the full marker set had significantly higher model R^2 than those using the common marker set (Fig. 6A) and the predictive models in the EV population had higher model R^2 than those in the SU and BM ($p < 0.001$) (Fig. 6B), a finding consistent with the results of r_{MP}.

4. Discussion

4.1. Estimation of broad-sense heritability in MAD2 trials

H^2 is the ratio of genetic to total phenotypic variance, representing the extent to which studied genotypes are affected by environment and experimental error and the accuracy or repeatability of phenotypic selection in breeding.

Table 4 – Correlation coefficients between r_E and broad-sense heritability ($\widehat{H}^2$) estimated using different methods.

	$\widehat{H}^2$(AM)	$\widehat{H}^2$(AP)	$\widehat{H}^2$(RM)	$\widehat{H}^2$(RP)
r_E	0.8164	0.9799	0.8857	0.9943
$\widehat{H}^2$(AM)		0.7819	0.9591	0.8031
$\widehat{H}^2$(AP)			0.8211	0.9898
$\widehat{H}^2$(RM)				0.8616

The sample size for correlation is 15 (three populations and five traits). r_E: mean value of correlation coefficients of trait performance between all possible pairs of environments; $\widehat{H}^2$(AM): $\widehat{H}^2$ estimated by ANOVA on an entry-mean basis; $\widehat{H}^2$(AP): $\widehat{H}^2$ estimated by ANOVA on a plot basis; $\widehat{H}^2$(RM): $\widehat{H}^2$ estimated by REML on an entry-mean basis; $\widehat{H}^2$(RP): $\widehat{H}^2$ estimated by REML on a plot basis.

Heritability estimates were also critical for accurately estimating *RE* in this study. For a multi-environmental MAD2 trial to estimate H^2, the moment method based on joint ANOVA was proposed [75]. Recently [76,77], an alternative REML method has been widely used for estimation of heritability and genetic correlation. In addition, two types of H^2 estimates in plant breeding, on a plot basis and on an entry-mean basis, can be calculated. Because test lines have no replications in MAD2 trials, the H^2 estimates on a plot basis are suggested [75]. To assess the accuracy of H^2 estimates in MAD2 trials, all four methods (AM, AP, RM, and RP) were used to estimate H^2 for the five traits in the three populations. To determine the accuracy of the four methods, we first need to know the true H^2 values of the traits. Although the true H^2 values are unknown, it is possible to define an indicator that emulates the true H^2 values. Assuming that a trait has high heritability, the performance of lines in one environment should show high repeatability in other environments, that is, the trait performance of lines should have a high correlation between any pair of environments. In contrast, a low-heritability trait should show a low correlation of trait performance between any two environments. Accordingly, the mean value of the correlation coefficients between all possible pairs of environments in a multi-environmental trial (r_E) can be used as an indicator of the true H^2 value. A perfect linear relationship between the r_E values and the $\widehat{H}^2$ values calculated from different traits and populations is expected. The r_E and $\widehat{H}^2$ estimated from the four methods for the five traits in the three populations are shown in Table S3. Correlation coefficients among these $\widehat{H}^2$ and r_E were calculated (Table 4). Strong correlations of r_E with $\widehat{H}^2$ estimated using AP ($r = 0.9799$) and with $\widehat{H}^2$ estimated using RP ($r = 0.9943$) were observed (Table 4, Fig. 7). In addition, AP and RP yielded highly similar $\widehat{H}^2$ ($r = 0.9898$). However, both AM and RM overestimated H^2 (Fig. 7). These comparisons showed that ANOVA- and REML-based methods had highly similar H^2 estimates and that plot-based H^2 estimates were more accurate than entry-mean-based estimates in MAD2 trials. Accordingly, heritability estimates on a plot basis were used in this study.

4.2. Target traits in linseed breeding

Five major target traits in linseed breeding, OIL, IOD, LIO, LIN, and YLD, were evaluated in this study for genomic prediction. IOD is a measurement of lipid unsaturation and is calculated

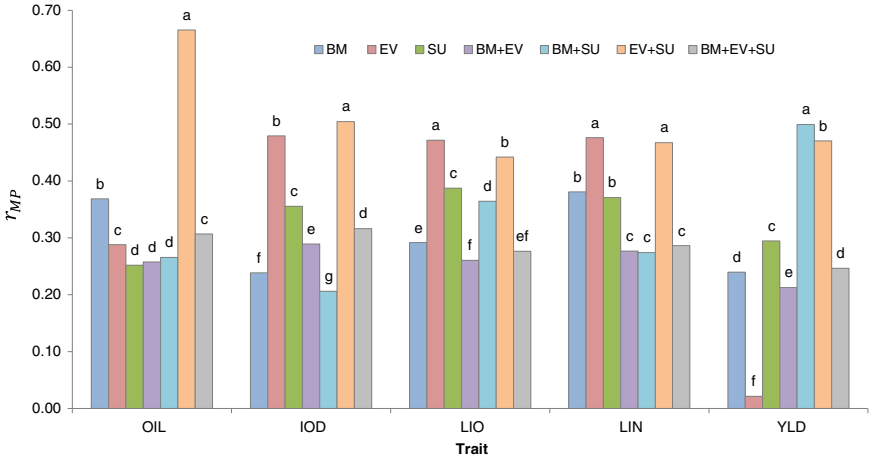

Fig. 5 – Comparison of prediction accuracies (r_{MP}) assessed using fivefold cross-validation and a common marker set for three single populations and their four pooled populations. The sizes of the training/test data sets were 194/47 for BM, 69/17 for EV, 56/14 for SU, 263/66 for BM + EV, 250/63 for BM + SU, 125/31 for EV + SU, and 319/80 for BM + EV + SU. For each trait, the same letters represent an absence of statistical significance at the 0.05 probability level. Duncan's multiple-range test was used.

from gas chromatography (GC)-derived fatty acid composition, with breeding lines with high LIN normally showing high IOD [53]. We observed highly significant genetic correlations $\hat{r}_G$ (close to 1 or −1) between IOD, LIO, and LIN in the three biparental populations, albeit different between IOD and LIO in BM (Table 2). The inverse correlations between IOD and LIO and between IOD and LIN in EV and SU are likely attributable to the inverse relationship of these two fatty acids in these populations as a consequence of the segregation of non-functional FAD3 enzymes, whereas the positive correlations in BM, a conventional cross with a much smaller range of variation in LIO and LIN content, reflect the smaller contribution to IOD of LIO with two double bonds than that of LIN with three. IOD has been an indicator and selective trait for favorable fatty acid composition in conventional flax breeding. Cloutier et al. (2010) [53] detected two major QTL for the colocated LIO, LIN, and IOD traits in SU, supporting the hypothesis of an interrelationship between these three traits.

In the randomly generated 100-sample sets (a total of 500 samplings because of fivefold cross-validation) of the same size as the training and test data sets, we observed that for different traits, the optimal training population with the maximum prediction accuracy within these samples was always different except for IOD, LIO, and LIN. We calculated the Pearson's correlation coefficients of r_{MP} between five traits using results from the 500 samplings and observed consistency between Pearson's correlation coefficients of r_{MP} and the genetic correlation coefficients among most traits (Table

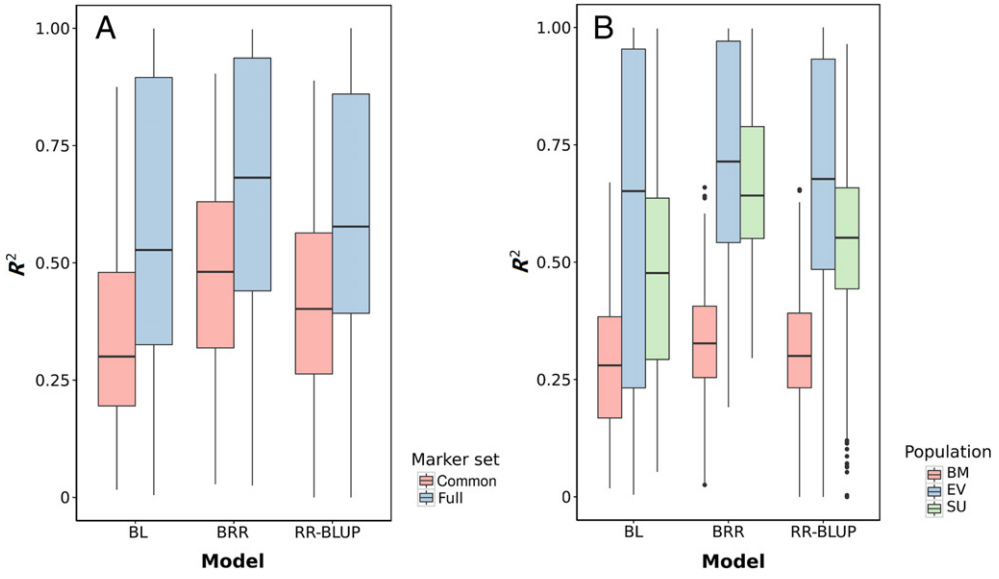

Fig. 6 – Box-and-whisker plots for the GS model R^2 assessed using fivefold cross-validation in three GS models in two marker sets (A) and three biparental populations (B).

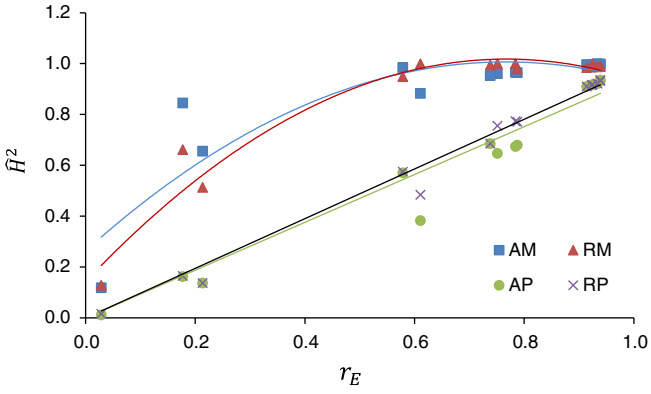

Fig. 7 – Relationship of r_E with broad-sense heritability ($\widehat{H}^2$) estimated by four methods. AM: ANOVA on an entry-mean basis; AP: ANOVA on a plot basis; RM: REML on an entry-mean basis; RP: REML on a plot basis. r_E is the average correlation coefficient of trait performance between all pairs of environments for a trait in a population.

2, lower triangle). Highly significant positive correlations of r_{MP} between IOD, LIO, and LIN (r = 0.93–0.99) were observed in EV and SU, showing that GS using any of these three traits will be effective in improving the other two as well. This result also further confirmed that the same QTL may contribute to the values of all three traits. Thus, IOD can be used as a representative trait for selection of high LIN or high LIO in linseed breeding.

Besides high LIN or high LIO, high YLD and high OIL are also important selection targets in linseed breeding. We did not find consistent and significant negative genetic correlations between YLD and OIL, LIN, or LIO in any of the populations (Table 2), a finding indicative of, among other things, the lack of genetic linkage among these traits. It is therefore possible to breed for individuals with high YLD, high OIL, and high LIN or high LIO from diverse breeding populations by crossing parental lines of large phenotypic differences in target traits.

4.3. r_{MP} and RE of GS

To evaluate the efficiency of GS for the five target traits in flax breeding, two criteria were used. r_{MP} based on a fivefold cross-validation scheme has been used to describe prediction accuracy [26,28,78,79] in many GS evaluation studies to date because of the linear relationship between prediction accuracy and genetic gain [80]. In fivefold cross-validation, any four folds (80%) of the individuals serve as a training population and the remaining fold (20%) is kept as a test data set. Usually, a large sampling of training and test data sets will be generated to estimate r_{MP}, generally resulting in a normal or nearly normal distribution of r_{MP} estimates. The mean or median of the distribution is used to represent the GS prediction accuracy of a trait [81]. In this study, the mean of r_{MP} estimates was used to represent the prediction accuracy under a certain training population size and at a combination of biparental populations, GS models, and marker sets. Our results showed r_{MP} for the five traits, ranging from 0.08 to 0.70 depending on traits, populations, and marker sets, comparable to those in biparental wheat populations of similar sizes [82,83] and in other crops [2,84].

RE represents a comparison for predictive response to one cycle of GS versus that to one cycle of phenotypic selection [20]. Generally, r_{MP} is related to the heritability of a trait. IOD, LIN, and LIO, with high heritability (Table 1), had high r_{MP} (0.50–0.56 for the full and 0.42–0.44 for the common marker set), whereas the r_{MP} of YLD was small (0.24 for both marker sets). Because of the higher heritability of IOD, LIN, and LIO, direct phenotypic selection on them would be more efficient than one cycle of GS. In contrast, GS for YLD had an overall mean *RE* of 1.52, showing significantly higher selection efficiency than direct phenotypic selection, whereas GS for IOD, LIN, and LIO had a low *RE* of 0.61–0.81 for the full marker set and 0.50–0.62 for the common marker set, being less efficient than direct phenotypic selection. However, in Canadian conversional flax breeding, one cycle of selection in the field and one generation for advance in a winter nursery can be achieved per year. In contrast, for GS, at least two cycles of selection can be applied in both the field season and winter nursery each year, allowing a doubling of the *RE* of GS. Even though the *RE*s for IOD, LIN, LIO, and OIL were less than 1.00, two cycles of GS per year for them are still more efficient than one cycle of phenotypic selection per year. In addition, the cost of phenotyping YLD and seed quality traits may exceed the cost of genotyping. Thus, GS for all five target traits could be efficient and could increase genetic gain per year and per unit cost.

4.4. GS models

Three GS models, RR-BLUP, BL, and BRR, were tested. Theoretically, the BRR method used here is the Bayesian equivalent of RR-BLUP [85]. Under the RR-BLUP model, marker effects have a fixed variance and all markers are included in the model by shrinkage of all marker effects to the same degree. It is assumed that a trait is controlled by multiple loci with small additive effects. Thus, RR-BLUP and BRR may be suitable for modeling complex traits such as seed yield and oil content. An empirical study in rice demonstrated that RR-BLUP was the best-performing method for grain yield where no large-effect QTL were detected by genome-wide association study [26]. BL, as a Bayesian model, assumes few

loci with large effects [72]. This assumption may fit traits controlled by several major genes or QTL such as the fatty acid composition in this study [86]. Because traits differ in their genetic architecture among and across populations, no single best model for all traits and populations exists, and the fit between the marker-effect assumptions and the genetic architecture of the target traits is thought [87–89] to be the most critical factor determining the effectiveness of GS models. Our results showed similar r_{MP} values, 0.39, 0.38, and 0.38 for BL, BRR, and RR-BLUP, respectively, as well as similar *RE* values for the three statistical models (0.72, 0.72, and 0.70, respectively). Compared with other factors (populations, marker sets, and traits), the GS model contributed the least to the mean squares in the joint ANOVA for r_{MP} and *RE* (Table S2), implying that selection of the GS model was less critical than that of populations, markers, and traits. RR-BLUP is the fastest method for computation and yields prediction accuracy superior or similar to that of other Bayesian methods [17,26,28,90]. From the standpoint of computational efficiency and prediction accuracy, RR-BLUP is the preferred predictive model for GS [17,26].

In this study, the additional statistic R^2 of a GS statistical model was adopted to describe the goodness of fit between markers and phenotypic data. It is noteworthy that the model R^2 of BRR was significantly higher than those of the other two models ($p < 0.001$), showing that markers in the BRR model may explain more genetic variation, possibly resulting in a higher r_{MP}.

4.5. *Markers*

Two sets of markers for each population, a full and a common marker set, were used in this study. The common marker set was the same for all three populations, whereas the full marker sets differed across populations, but all contained the common marker data set. The three full marker sets have been used to build the consensus genetic map of flax which covers an estimated 74% of the flax genome, showing that the full marker set includes a genome-wide set of markers [52]. We observed significant differences in r_{MP} between the full and the common marker sets, and observed significant interactions between marker sets and populations and between marker sets and traits (Table S2 and S3). Usually, higher r_{MP} was obtained for the full than for the common marker set, but YLD gave a higher r_{MP} for the common than for the full marker set. In some cases, we may need to include only markers linked to QTL, so that prior QTL information for traits may be useful [81]. On the other hand, the models of the full marker set explained a markedly larger portion of the total variation than those of the common marker set (Fig. 5A). On average, 65.6% of total variation for the full and 45.3% for the common marker set were explained by the models (data not shown). Although 340, 443, and 474 SSR markers distributed genome-wide in BM, EV, and SU, separately, were used for the GS evaluation, it appeared that the marker density was not sufficient for constructing more efficient models in flax. Additional genetic markers may be needed to increase the marker density and ultimately the proportion of the total trait variation explained by the models. With the rapid development of high-throughput genotyping techniques and the relatively low cost of genotyping-by-sequencing, it is feasible to genotype large populations with denser genetic markers such as single-nucleotide polymorphisms (SNPs) for further GS evaluation.

4.6. *Populations*

Biparental multi-families are the major breeding selection populations in self-fertilizing crops including flax. Evaluation of GS accuracy using such populations is warranted for further practical GS. Some studies of genomic selection using biparental populations have been reported in other crop plants such as wheat, maize, *Arabidopsis*, and barley [4,14,79,82,91] or using simulation data [92]. In our study, three biparental populations were used for evaluation of GS. Significant differences in r_{MP} in both marker sets of the three biparental populations ($p < 0.001$) were observed (Tables S2 and 3). These differences are resulted mostly from different architectures and genetic variation of the three biparental populations rather than differences in SSR markers, given that the three populations shared the same SSR markers in the common marker set. The PCA (Fig. 2) and estimates of genetic parameters ($\hat{\sigma}^2_G$, $\widehat{GCV}$, and $\hat{r}_G$) of the three populations (Tables 1 and 2) revealed these marked between-population differences in genetic architecture and variation. An increase of r_{MP} and *RE* in flax will rely heavily on the assessment of diverse populations, as shown by other studies [87–89,92]. These results show that similarity of genetic architecture between training and test populations is needed for efficient GS. Because of the limited genetic base in single biparental populations, an elite, diverse germplasm population containing ancestors and elite direct parents of modern flax cultivars would be a good choice for a training population used to construct a predictive model, because this type of population possesses the core genetic base for the breeding lines developed in breeding programs [39].

The size of the training population is another important factor in GS. The sizes of the three biparental populations in this study were 243, 86, and 70 for BM, EV, and SU, respectively (Table 1). Because of the fivefold cross-validation approach, the actual sizes of the training populations were 194, 69, and 56 for BM, EV, and SU, respectively. We observed a wide distribution of prediction accuracies among the 500 randomly sampled training populations [27]. The accuracy values ranged from large negatives to large positives. The negative r_{MP} may result from distinct genetic architectures between the training and the test data sets sampled from small breeding populations. As the size of the training population increases, the mean and maximum r_{MP} estimates in the randomly sampled populations will increase and the standard deviation will decrease [14,27,82]. Although BM had a training population size of 194, much larger than those of EV and SU, the r_{MP} and *RE* of the five traits in BM were significantly lower than those in EV and SU (Table 3), most likely owing to the low genetic variation of the traits. In some of the four pooled populations that contained two or three single biparental populations (Fig. 5), the r_{MP} significantly increased for the studied traits, especially those with low heritability such as YLD. However, the increase in the training population size and genetic diversity in some pooled populations did not

always make the GS more efficient (Fig. 5). Thus, the size and genetic diversity of a training population may play a comprehensive role in model construction. Further studies are needed to determine the effective size and genetic architecture of an optimal training population in flax.

5. Conclusion

Using two (full and common) sets of SSR markers in each of three biparental populations or their pooled populations for five target traits in linseed breeding, three GS models (RR-BLUP, BL, and BRR) were evaluated. The three GS models showed similar r_{MP} and *RE*; however, BRR yielded higher R^2 of fitted models than RR-BLUP and BL. The fitted GS models using the full SSR marker sets explained more variation than those using the common marker set. Traits such as IOD, LIO, and LIN, with higher genetic variation and heritability in a population, had higher r_{MP} than low-heritability traits such as YLD and OIL, whereas YLD showed an overall mean *RE* of 1.52 across all populations and two marker sets. GS for all five traits may outperform direct phenotypic selection if two or more cycles of GS per year are conducted. The empirical results demonstrate that GS could increase genetic gain per unit time for linseed breeding, but further studies are needed to build optimal training populations and marker sets for application of GS in linseed breeding.

Acknowledgments

This work was conducted as part of the A-base project (No. 1142) funded by Agriculture and Agri-Food Canada, the Total Utilization Flax GENomics (TUFGEN) project funded by Genome Canada and other stakeholders, and the flax breeding database project funded by Western Grain Research Foundation (WGRF). The authors thank Andrzej Walichnowski for manuscript editing.

Supplementary data

Supplementary data for this article can be found online at http://dx.doi.org/10.1016/j.cj.2016.03.001.

REFERENCES

[1] E. Jonas, D.J. de Koning, Does genomic selection have a future in plant breeding? Trends Biotechnol. 31 (2013) 497–504.
[2] A. Nakaya, S.N. Isobe, Will genomic selection be a practical method for plant breeding? Ann. Bot. 110 (2012) 1303–1316.
[3] T.H. Meuwissen, B.J. Hayes, M.E. Goddard, Prediction of total genetic value using genome-wide dense marker maps, Genetics 157 (2001) 1819–1829.
[4] E.L. Heffner, J.L. Jannink, M.E. Sorrells, Genomic selection accuracy using multifamily prediction models in a wheat breeding program, Plant Genome 4 (2011) 65–75.
[5] N. Piyasatian, R.L. Fernando, J.C. Dekkers, Genomic selection for marker-assisted improvement in line crosses, Theor. Appl. Genet. 115 (2007) 665–674.
[6] R. Berbardo, J. Yu, Prospects for genomewide selection for quantitative trait in maize, Crop Sci. 47 (2007) 1082–1090.
[7] P.J. Mayor, R. Berbardo, Genomewide selection and marker-assisted recurrent selection in doubled haploid versus F_2 populations, Crop Sci. 49 (2009) 1719–1725.
[8] R. Bernardo, Genomewide selection for rapid introgression of exotic germplasm in maize, Crop Sci. 49 (2009) 419–425.
[9] C.K. Wong, R. Bernardo, Genomewide selection in oil palm: increasing selection gain per unit time and cost with small populations, Theor. Appl. Genet. 116 (2008) 815–824.
[10] R. Bernardo, Genomewide selection with minimal crossing in self-pollinated crops, Crop Sci. 50 (2010) 624–627.
[11] H. Iwata, J.L. Jannink, Accuracy of genomic selection prediction in barley breeding programs: a simulation study based on the real single nucleotide polymorphism data of barley breeding lines, Crop Sci. 51 (2011) 1915–1927.
[12] S.Q. Zhong, J.C. Dekkers, R.L. Fernando, J.L. Jannink, Factors affecting accuracy from genomic selection in populations derived from multiple inbred lines: a barley case study, Genetics 182 (2009) 355–364.
[13] J.L. Jannink, Dynamics of long-term genomic selection, Genet. Sel. Evol. 42 (2010) 35.
[14] R.E. Lorenzana, R. Bernardo, Accuracy of genotypic value predictions for marker-based selection in biparental plant populations, Theor. Appl. Genet. 120 (2009) 151–161.
[15] H.P. Piepho, Ridge regression and extensions for genomewide selection in maize, Crop Sci. 49 (2009) 1165–1176.
[16] J. Crossa, L. Campos Gde, P. Perez, D. Gianola, J. Burgueno, J.L. Araus, D. Makumbi, R.P. Singh, S. Dreisigacker, J. Yan, V. Arief, M. Banziger, H.J. Braun, Prediction of genetic values of quantitative traits in plant breeding using pedigree and molecular markers, Genetics 186 (2010) 713–724.
[17] Z.G. Guo, D.M. Tucker, J.W. Lu, V. Kishore, G. Gay, Evaluation of genome-wide selection efficiency in maize nested association mapping populations, Theor. Appl. Genet. 124 (2011) 261–275.
[18] J. Crossa, Y. Beyene, S. Kassa, P. Perez, J.M. Hickey, C. Chen, G. de los Campos, J. Burgueno, V.S. Windhausen, E. Buckler, J.L. Jannink, M.A. Lopez Cruz, R. Babu, Genomic prediction in maize breeding populations with genotyping-by-sequencing, Genes Genom. Genet. 3 (2013) 1903–1926.
[19] J. Crossa, P. Perez, J. Hickey, J. Burgueno, L. Ornella, J. Ceron-Rojas, X. Zhang, S. Dreisigacker, R. Babu, Y. Li, D. Bonnett, K. Mathews, Genomic prediction in CIMMYT maize and wheat breeding programs, Heredity 112 (2013) 48–60.
[20] C. Ziyomo, R. Bernardo, Drought tolerance in maize: indirect selection through secondary traits versus genomewide selection, Crop Sci. 53 (2013) 1269–1275.
[21] L.M. Krchov, R. Bernardo, Relative efficiency of genomewide selection for testcross performance of doubled haploid lines in a maize breeding program, Crop Sci. 55 (2015) 2091–2099.
[22] E. Combs, R. Bernardo, Accuracy of genomewide selection for different traits with constant population size, heritability, and number of markers, Plant Genome 6 (2013) 1–7.
[23] M.P. Mendes, C.L. Souza Jr., Genomewide prediction of tropical maize single-crosses, Euphytica (2016), http://dx.doi.org/10.1007/s10681-016-1642-1.
[24] V. Wimmer, C. Lehermeier, T. Albrecht, H.J. Auinger, Y. Wang, C.C. Schon, Genome-wide prediction of traits with different genetic architecture through efficient variable selection, Genetics 195 (2013) 573–587.
[25] S. Xu, Genetic mapping and genomic selection using recombination breakpoint data, Genetics 195 (2013) 1103–1115.
[26] J. Spindel, H. Begum, D. Akdemir, P. Virk, B. Collard, E. Redona, G. Atlin, J.L. Jannink, S.R. McCouch, Genomic selection and association mapping in rice (*Oryza sativa*): effect of trait genetic architecture, training population composition, marker number and statistical model on

accuracy of rice genomic selection in elite, tropical rice breeding lines, PLoS Genet. 11 (2015), e1004982.
[27] T. Wurschum, J.C. Reif, T. Kraft, G. Janssen, Y. Zhao, Genomic selection in sugar beet breeding populations, BMC Genet. 14 (2013) 85.
[28] M. Gouy, Y. Rousselle, D. Bastianelli, P. Lecomte, L. Bonnal, D. Roques, J.C. Efile, S. Rocher, J. Daugrois, L. Toubi, S. Nabeneza, C. Hervouet, H. Telismart, M. Denis, A. Thong-Chane, J.C. Glaszmann, J.Y. Hoarau, S. Nibouche, L. Costet, Experimental assessment of the accuracy of genomic selection in sugarcane, Theor. Appl. Genet. 126 (2013) 2575–2586.
[29] D. Grattapaglia, M.D. Resende, M.R. Resende, C.P. Sansaloni, P.C.D., A.A. Missiaggia, E.K. Takahashi, K.C. Zamprogno, A. Kilian, Genomic selection for growth traits in *Eucalyptus*: accuracy within and across breeding populations, BMC Proc. 5 (Suppl. 7) (2011) O16.
[30] F. Isik, R. Whetten, J. Zapata-Valenzuela, F. Ogut, S. Mckeand, Genomic selection in loblolly pine–from lab to field, BMC Proc. 5 (Suppl. 7) (2011) 18.
[31] M.F.R. Resende, P.R.M. Del Valle, J.J. Acosta, M.D.V. Resende, D. Grattapaglia, M. Kirst, Stability of genomic selection prediction models across ages and environments, BMC Proc. 5 (2011) O14.
[32] S. Kumar, D. Chagné, M.C.A.M. Bink, R.K. Volz, C. Whitworth, C. Carlisle, Genomic selection for fruit quality traits in apple (*Malus*× *domestica* Borkh.), PLoS One 7 (2012) e36674.
[33] B. Soto-Cerda, A. Diederichsen, R. Ragupathy, S. Cloutier, Genetic characterization of a core collection of flax (*Linum usitatissimum* L.) suitable for association mapping studies and evidence of divergent selection between fiber and linseed types, BMC Plant Biol. 13 (2013) 78.
[34] N.D. Westcott, N.D. Muir, Chemical studies on the constituents of *Linum* sp, in: A.D. Muir, N.D. Westcott (Eds.), Flax, The Genus *Linum*, CRC Press, Boca Raton 2003, pp. 55–73.
[35] A. Diederichsen, P.M. Kusters, D. Kessler, Z. Bainas, R.K. Gugel, Assembling a core collection from the flax world collection maintained by Plant Gene Resources of Canada, Genet. Resour. Crop. Evol. 60 (2013) 1479–1485.
[36] W. Demark-Wahnefried, Flaxseed and Its Potential for Cancer Control and Personalized Medicine, Proceedings of the 65th Flax Institute of the United States, Fargo, North Dakota, 2014.
[37] Health Canada, Summary of Health Canada's Assessment of a Health Claim about Ground Whole Flaxseed and Blood Cholesterol Lowering, 2014.
[38] S.E. Rickard-Bon, L.U. Thompson, The role of flaxseed lignans in hormone-dependant and independent cancer, in: A.D. Muir, N.D. Westcott (Eds.), Flax, The Genus *Linum*, CRC Press, Boca Raton 2003, pp. 181–203.
[39] F.M. You, S.D. Duguid, I. Lam, S. Cloutier, K.Y. Rashid, H. Booker, Pedigrees and genetic base of the flax varieties registered in Canada, Can. J. Plant Sci. 95 (2016) (in press).
[40] A.G. Green, A mutant genotype of flax (*Linum usitatissimum* L.) containing very low levels of linolenic acid in its seed oil, Can. J. Plant Sci. 66 (1986) 499–503.
[41] G.G. Rowland, An EMS-induced low-linolenic-acid mutant in McGregor flax (*Linum usitatissimum* L.), Can. J. Plant Sci. 71 (1991) 393–396.
[42] S.C. Cunnane, S. Ganguli, C. Menard, A.C. Liede, M.J. Hamadeh, Z.Y. Chen, T.M. Wolever, D.J. Jenkins, High α-linolenic acid flaxseed (*Linum usitatissimum*): some nutritional properties in humans, Brit. J. Nutr. 69 (1993) 443–453.
[43] A.G. Green, S.P. Singh, Y. Chen, J.C.P. Dribnenki, Flax, Compend. Transgenic Crop Plants 2 (5) (2009) 199–226.
[44] W. Powell, G.C. Machray, J. Provan, Polymorphism revealed by simple sequence repeats, Trends Plant Sci. 1 (1996) 215–222.
[45] S. Cloutier, E. Miranda, K. Ward, N. Radovanovic, E. Reimer, A. Walichnowski, R. Datla, G. Rowland, S. Duguid, R. Ragupathy, Simple sequence repeat marker development from bacterial artificial chromosome end sequences and expressed sequence tags of flax (*Linum usitatissimum* L.), Theor. Appl. Genet. 125 (2012) 685–694.
[46] S. Cloutier, Z. Niu, R. Datla, S. Duguid, Development and analysis of EST-SSRs for flax (*Linum usitatissimum* L.), Theor. Appl. Genet. 119 (2009) 53–63.
[47] B. Soto-Cerda, R. Carrasco, G. Aravena, H. Urbina, C. Navarro, Identifying novel polymorphic microsatellites from cultivated flax (*Linum usitatissimum* L.) following data mining, Plant Mol. Biol. Report. 29 (2011) 753–759.
[48] C.L. Bickel, S. Gadani, M. Lukacs, C.A. Cullis, SSR markers developed for genetic mapping in flax (*Linum usitatissimum* L.), Res. Rep. Biol. (2011) 23–29.
[49] X. Deng, S.H. Long, D.F. He, X. Li, Y.F. Wang, D.M. Hao, C.S. Qiu, X.B. Chen, Isolation and characterization of polymorphic microsatellite markers from flax (*Linum usitatissimum* L.), Afr. J. Biotechnol. 10 (2011) 734–739.
[50] X. Deng, S.H. Long, D.F. He, X. Li, Y.F. Wang, J. Liu, X.B. Chen, Development and characterization of polymorphic microsatellite markers in *Linum usitatissmum*, J. Plant Res. 123 (2010) 119–123.
[51] C. Roose-Amsaleg, E. Cariou-Pham, D. Vautrin, R. Tavernier, M. Solignac, Polymorphic microsatellite loci in *Linum usitatissimum*, Mol. Ecol. Notes 6 (2006) 796–799.
[52] S. Cloutier, R. Ragupathy, E. Miranda, N. Radovanovic, E. Reimer, A. Walichnowski, K. Ward, G. Rowland, S. Duguid, M. Banik, Integrated consensus genetic and physical maps of flax (*Linum usitatissimum* L.), Theor. Appl. Genet. 125 (2012) 1783–1795.
[53] S. Cloutier, R. Ragupathy, Z. Niu, S. Duguid, SSR-based linkage map of flax (*Linum usitatissimum* L.) and mapping of QTLs underlying fatty acid composition traits, Mol. Breed. 28 (2010) 437–451.
[54] B.J. Soto-Cerda, I. Maureira-Butler, G. Munoz, A. Rupayan, S. Cloutier, SSR-based population structure, molecular diversity and linkage disequilibrium analysis of a collection of flax (*Linum usitatissimum* L.) varying for mucilage seed-coat content, Mol. Breed. 30 (2012) 875–888.
[55] Y.B. Fu, G.W. Peterson, Characterization of expressed sequence tag-derived simple sequence repeat markers for 17 *Linum* species, Botany 88 (2010) 537–543.
[56] B.J. Soto-Cerda, S. Duguid, H. Booker, G. Rowland, A. Diederichsen, S. Cloutier, Association mapping of seed quality traits using the Canadian flax (*Linum usitatissimum* L.) core collection, Theor. Appl. Genet. 127 (2014) 881–896.
[57] B.J. Soto-Cerda, S. Duguid, H. Booker, G. Rowland, A. Diederichsen, S. Cloutier, Genomic regions underlying agronomic traits in linseed (*Linum usitatissimum* L.) as revealed by association mapping, J. Integr. Plant Biol. 56 (2013) 75–87.
[58] G.G. Rowland, Y.A. Hormis, K.Y. Rashid, CDC Bethune flax, Can. J. Plant Sci. 82 (2002) 101–102.
[59] S.D. Duguid, E.O. Kenaschuk, K.Y. Rashid, Macbeth flax, Can. J. Plant Sci. 83 (2003) 803–805.
[60] G.G. Rowland, R.S. Bhatty, Ethyl meththane-sulphonate induced fatty acid mutations in flax, J. Am. Oil Chem. Soc. 67 (1990) 213–214.
[61] M. Banik, S. Duguid, S. Cloutier, Transcript profiling and gene characterization of three fatty acid desaturase genes in high, moderate, and low linolenic acid genotypes of flax (*Linum usitatissimum* L.) and their role in linolenic acid accumulation, Genome 54 (2011) 471–483.
[62] S. Kumar, F.M. You, S. Duguid, H. Booker, G. Rowland, S. Cloutier, QTL for fatty acid composition and yield in linseed (*Linum usitatissimum* L.), Theor. Appl. Genet. 128 (2015) 965–984.
[63] C.S. Lin, G. Poushinsky, A modified augmented design (type 2) for rectangular plots, Can. J. Plant Sci. 65 (1985) 743–749.
[64] F.M. You, S.D. Duguid, D. Thambugala, S. Cloutier, Statistical analysis and field evaluation of the type 2 modified

augmented design (MAD) in phenotyping of flax (*Linum usitatissimum*) germplasms in multiple environments, Aust. J. Crop. Sci. 7 (2013) 1789–1800.
[65] J.K. Daun, P.B. Mazur, Use of gas liquid chromatography for monitoring the fatty acid composition of Canadian rapeseed, J. Am. Oil Chem. Soc. 60 (1983) 1751–1754.
[66] J.B. Endelman, Ridge regression and other kernels for genomic selection with R package rrBLUP, Plant Genome 4 (2011) 250–255.
[67] J. Poland, J. Endelman, J. Dawson, J. Rutkoski, S. Wu, Y. Manes, S. Dreisigacker, J. Crossa, H. Sánchez-Villeda, M. Sorrells, J.L. Jannink, Genomic selection in wheat breeding using genotyping-by-sequencing, Plant Genome 5 (2012) 103–113.
[68] J.C. Whittaker, R. Thompson, M.C. Denham, Marker-assisted selection using ridge regression, Genet. Res. 75 (2000) 249–252.
[69] T. Park, G. Casella, The Bayesian Lasso, J. Am. Stat. Assoc. 103 (2008) 681–686.
[70] G. de los Campos, H. Naya, D. Gianola, J. Crossa, A. Legarra, E. Manfredi, K. Weigel, J.M. Cotes, Predicting quantitative traits with regression models for dense molecular markers and pedigree, Genetics 182 (2009) 375–385.
[71] G. de los Campos, J.M. Hickey, R. Pong-Wong, H.D. Daetwyler, M.P.L. Calus, Whole-genome regression and prediction methods applied to plant and animal breeding, Genetics 193 (2013) 327–345.
[72] A.J. Lorenz, S. Chao, F.G. Asoro, E.L. Heffner, T. Hayashi, H. Iwata, K.P. Smith, M.E. Sorrells, J.L. Jannink, Genomic selection in plant breeding: knowledge and prospects, Adv. Agron. 110 (2011) 77–123.
[73] G. de los Campos, P. Perez, A.I. Vazquez, J. Crossa, Genome-enabled prediction using the BLR (Bayesian Linear Regression) R-package, in: C. Gondro, J. van der Werf, B. Hayes (Eds.), Genome-Wide Association Studies and Genomic Prediction, Methods in Molecular Biology, 1019, Humana Press, New York 2013, pp. 299–320.
[74] J.C. Dekkers, Prediction of response to marker-assisted and genomic selection using selection index theory, J. Anim. Breed. Genet. 124 (2007) 331–341.
[75] F.M. You, Q. Song, G. Jia, Y. Cheng, S. Duguida, H. Booker, S. Cloutierd, Estimation of genetic parameters and their sampling variances for quantitative traits in the type 2 modified augmented design, Crop J. 4 (2016) 107–118.
[76] J.B. Holland, Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation with SAS Proc MIXED, Crop Sci. 46 (2006) 642–654.
[77] H.P. Piepho, J. Mohring, On estimation of genotypic correlations and their standard errors by multivariate REML using the MIXED procedure of the SAS system, Crop Sci. 51 (2011) 2449–2454.
[78] Z. Guo, D.M. Tucker, C.J. Basten, H. Gandhi, E. Ersoz, B. Guo, Z. Xu, D. Wang, G. Gay, The impact of population structure on genomic prediction in stratified populations, Theor. Appl. Genet. 127 (2014) 749–762.
[79] A. Jaconson, L. Lian, S. Zhong, R. Bernardo, Marker imputation before genomewide selection in biparental maize populations, Plant Genome 8 (2015) 1220–1235.
[80] E.L. Heffner, A.J. Lorenz, J.L. Jannink, M.E. Sorrells, Plant breeding with genomic selection: gain per unit time and cost, Crop Sci. 50 (2010) 1681–1690.
[81] J. Rutkoski, J. Benson, Y. Jia, G. Brown-Guedira, J.L. Jannink, M.E. Sorrells, Evaluation of genomic prediction methods for Fusarium head blight resistance in wheat, Plant Genome 5 (2012) 51–61.
[82] E.L. Heffner, J.L. Jannink, H. Iwata, E. Souza, M.E. Sorrells, Genomic selection accuracy for grain quality traits in biparental wheat populations, Crop Sci. 51 (2011) 2597–2606.
[83] J. Crossa, P. Pérez, G. de los Campos, G. Mahuku, S. Dreisigacker, C. Magorokosho, Genomic selection and prediction in plant breeding, J. Crop Improv. 25 (2011) 239–261.
[84] Z. Lin, B.J. Hayes, H.D. Daetwyler, Genomic selection in crops, trees and forages: a review, Crop Pasture Sci. 65 (2014) 1177–1191.
[85] G. de los Campos, J.M. Hickey, R. Pong-Wong, H.D. Daetwyler, M.P. Calus, Whole-genome regression and prediction methods applied to plant and animal breeding, Genetics 193 (2013) 327–345.
[86] F.M. You, P. Li, S. Kumar, R. Ragupathy, Z. Li, Y.B. Fu, S. Cloutier, Genome-wide identification and characterization of the gene families controlling fatty acid biosynthesis in flax (*Linum usitatissimum* L.), J. Proteom. Bioinform. 7 (2014) 310–326.
[87] S.A. Clark, J.M. Hickey, J.H.J. Van der Werf, Different models of genetic variation and their effect on genomic evaluation, Genet. Sel. Evol. 43 (2011) 18.
[88] H.D. Daetwyler, R. Pong-Wong, B. Villanueva, J.A. Woolliams, The impact of genetic architecture on genome-wide evaluation methods, Genetics 185 (2010) 1021–1031.
[89] Y. Zhao, J. Zeng, R. Fernando, J.C. Reif, Genomic prediction of hybrid wheat performance, Crop Sci. 53 (2013) 802–810.
[90] G. Moser, B. Tier, R.E. Crump, M.S. Khatkar, H.W. Raadsma, A comparison of five methods to predict genomic breeding values of dairy bulls from genome-wide SNP markers, Genet. Sel. Evol. 41 (2009) 56.
[91] C. Riedelsheimer, J.B. Endelman, M. Stange, M.E. Sorrells, J.L. Jannink, A.E. Melchinger, Genomic predictability of interconnected biparental maize populations, Genetics 194 (2013) 493–503.
[92] J.J. Marulanda, A.E. Melchinger, T. Würschum, Genomic selection in biparental populations: assessment of parameters for optimum estimation set design, Plant Breed. 134 (2015) 623–630.